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Some Aspects of the Life History and Ecology of the Pitch Nodule Maker, *Petrova albicapitana* (Busck) (Lepidoptera-Olethreutidae)¹

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Introduction

The widespread presence of the pitch nodule maker in pine plantations and jack-pine regeneration in Manitoba and Saskatchewan stimulated this investigation. Although this insect is not causing serious damage at present it could become a problem should the area of pine plantations be increased in Manitoba and Saskatchewan.

History and Distribution of *Petrova albicapitana* in North America

The insect was first described by Busck (1) in 1914 as *Evetria albicapitana*. Caesar (4) reported it to be abundant on jack-pine plantations at St. Williams, Ontario, in 1917. *Evetria albicapitana* was placed in the new genus *Petrova* of the subfamily Eucosminae by Heinrich (6) in 1923. In 1924, de Gryse (5) reported *P. albicapitana* to be of some importance in pine plantations of the Prairie Provinces of Canada. Pettit (8) observed that this species was common in Michigan in 1927.

In preparing the following description of the distribution of *P. albicapitana* information was obtained chiefly from the records of the Canadian Forest Insect Survey, supplemented by reports of the United States National Museum, the Minnesota Forest Insect Survey, and miscellaneous publications.

The distribution of the pitch nodule maker in relation to its hosts is shown in Figure 1. The distribution of jack pine and lodgepole pine was taken from Native Trees of Canada, fourth edition, 1949, and Trees, U.S. Department of Agriculture Yearbook for 1949. With the exception of unsurveyed areas, *P. albicapitana* has been reported from all parts of North America where jack pine, *Pinus Banksiana* Lamb., occurs naturally. In the west, reports of *P. albicapitana* on lodgepole pine, *Pinus contorta* Dougl. var. *latifolia* Engelm., are limited to the eastern slope of the Rocky Mountains and the Cypress Hills in Alberta, Corlett, Wyoming, and Coeur d'Alene, Idaho. Keen (7) reports this insect on *Pinus ponderosa* Laws. in the Western United States.

Plantations of jack pine, lodgepole pine, and Scots pine, *Pinus sylvestris* L., are heavily attacked. Outside the ranges of the native hosts damage has been reported from plantations in the St. Lawrence Valley, Quebec; southern Ontario; Ann Arbor, Michigan; Anoka, Minnesota; Winnipeg, Selkirk and Spruce Woods Forest Reserve, Manitoba; and Regina, Saskatchewan.

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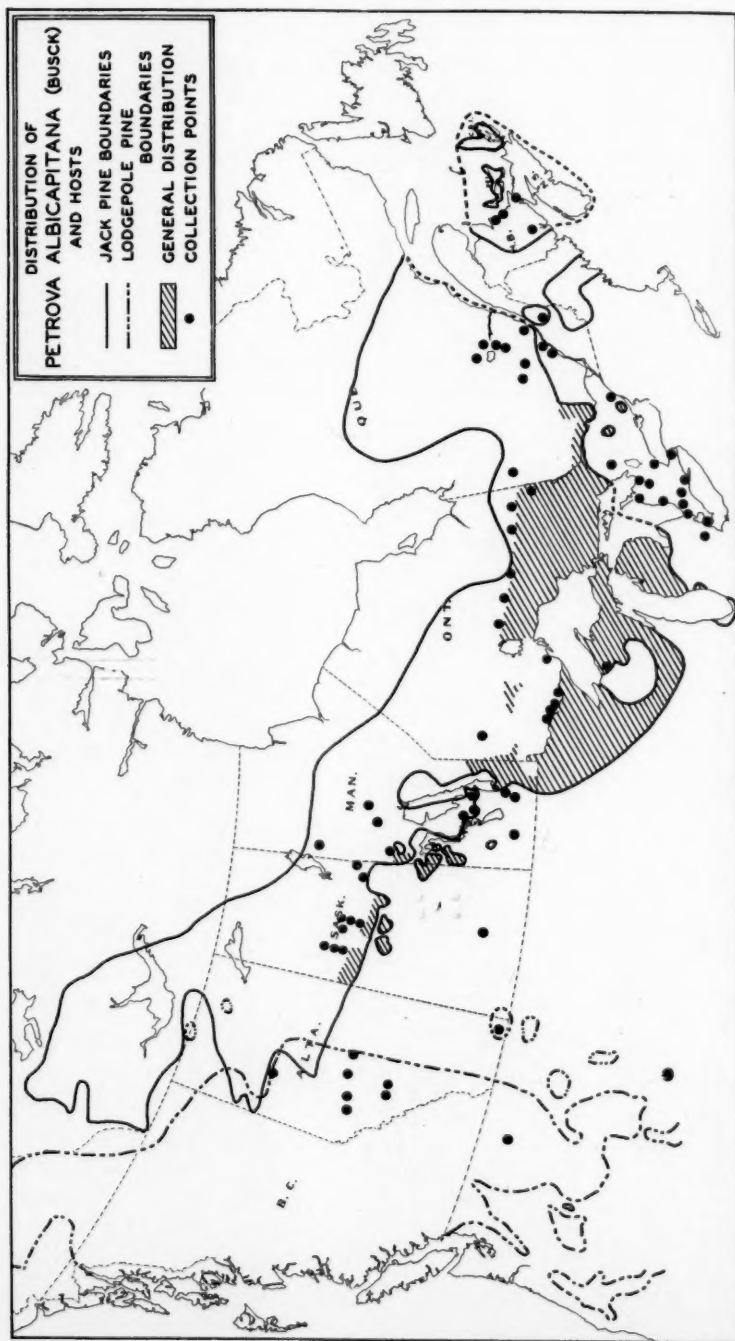


Fig. 1 Distribution of *Petrova albicapitana* in North America.

Life History of *Petrova albicapitana* (Busck)

The data on the life history of this species were accumulated at intervals during the summers of 1948, 1949, and 1950 at several localities in Manitoba and Saskatchewan, and at Cass Lake, Minnesota, in June 1950.

The insect requires two years to complete its life cycle. The eggs are laid from early June to mid-July. The young larvae feed in small nodules until cold weather occurs and then become dormant. In spring the larvae become active and feed until the next winter, which is also passed in the larval stage. In early spring of the third year, the larvae feed for a short time and then pupate. Adults may emerge from early June to mid-July, depending on seasonal variation.

Oviposition and Egg Stage

The oviposition behaviour of a single adult female of *P. albicapitana* was observed under natural conditions at Brainerd, Minnesota, on June 14, 1950. The new needles of jack pine had emerged one-eighth to three-sixteenths of an inch from the sheath. At 2000 hours, C.S.T., when the light was fading rapidly and the temperature was about 70°F., oviposition was observed. The adult alighted on a terminal shoot, moved to the tip, and deposited a single egg at the base of a needle sheath. Caesar (4) described the eggs laid by caged females as being laid in overlapping clusters on a needle. The unnatural conditions and lack of suitable oviposition sites might have prevented normal oviposition.

The eggs of *P. albicapitana* are tear-shaped in outline, somewhat flattened, lemon-yellow in colour, and approximately 0.5 mm. in diameter.

Larval Stage

Each newly-hatched first-instar larva apparently begins to feed on the succulent terminal growth at a point very close to the oviposition site. By early September the larva has formed a small circular excavation in the cortex of the host tissue and covered it with a layer of silk and pitch (Fig. 2). The young larva feeds within this structure until late fall. The larva passes the first winter in this nodule. In the spring, the larva resumes feeding on the cortex, enlarging the previous excavation. By feeding, the larva produces a nodule about the same size and shape as the overwintering nodule.

About the beginning of June most of the larvae leave their overwintering nodules and move proximally along the branch. Each larva resumes its feeding at a crotch on the main stem or branch, and forms a new nodule at this point. The average distance moved by the larvae was found to be 15 inches; the maximum distance moved by any one larva was 54 inches.

When the larva begins to feed in the new location, it spins a silken cover about three-eighths of an inch in diameter over the proposed feeding site. Resin flowing from the injured host tissues, and frass and more silk from the larva, are added to this to form the nodule. The inner surface of the nodule is lined with silk. The larva feeds on the bark, cambium, and outer xylem of the host. The nodule attains a diameter of about three-quarters of an inch and a thickness of one-quarter of an inch by the end of the summer (Fig. 3). The larva becomes dormant about mid-October and remains in the nodule throughout the winter.

During the last week of April or the first week of May of the third season, the larva becomes active and feeds for two or three weeks. The inner structure of the nodule is modified to provide a thick-walled pupal chamber with a silk lining. One end of this chamber is closed by only a thin layer of silk and pitch. The prepupal period appears to be very short. In Manitoba, pupation usually occurs shortly after May 15, although in 1950 many larvae did not pupate until the second week of June.



Fig. 2. First year nodule in October (D. Anderson).
Fig. 3. Second year nodule in October (B. McLeod).

Pupal Stage

The pupa of *P. albicapitana* was described by Butcher (2). The duration of the pupal stage is about three weeks under natural conditions. Larvae collected at Spruce Woods Forest Reserve, Manitoba, in early May, 1948, pupated between May 17 and May 28 in the insectary at Winnipeg. The mean length of the pupal period was 20 days; 19 days for males and 22 days for females.

Adult Emergence

The records of the Forest Insect Survey rearings of *P. albicapitana* for 1948, 1949, and 1950, were used to delimit the period of adult emergence. Only the records of collections from southern Manitoba were used. The specimens were kept in closed jars in the insectary. Adults emerged from June 4 to 24 in 1948, from June 1 to 24 in 1949, and from June 14 to July 14 in 1950. The sex ratio among these reared adults was approximately 1:1.

Because of the two-year life cycle there are two broods of *P. albicapitana* present in each locality, usually of unequal size. Of twenty stands that were examined in Manitoba and Saskatchewan only two had broods of equal size.

Description and Significance of Injury

Characteristics of Injury

The larvae of *P. albicapitana* are responsible for two types of injury. The small first-year larvae feed on the cortex of the growing terminal shoots and occasionally girdle and destroy the tip of small unthrifty terminals. Most of the larval feeding takes place during the second summer, after the year-old larva has migrated to form a new nodule at the crotch formed by some branch.

Table I indicates the parts of the trees that are most commonly attacked by the larvae of *P. albicapitana* before and after migration of the year-old larvae in June. The first-year nodules are situated predominantly on the laterals, probably because of their relative abundance. The migrating larvae apparently prefer to



Fig. 4. Leader killed by second year larva (R. D. Bird).

Fig. 5. Distorted main stem caused by injury from second year larva (R. D. Bird).

resume feeding on the main stem. This preference appears to be greater among larvae on smaller trees and may be conditioned by the limited availability of feeding sites on branches.

TABLE I

Per Cent of Nodules of *P. albicapitana* on Different Parts of Jack-pine Seedlings before and after the Migration of Year-old Larvae in June, 1950.

Location of nodules	Cass Lake, Minnesota		Spruce Woods Forest Reserve, Manitoba	
	Before migration	After migration	Before migration	After migration
Leader.....	15.6	60.0	10.5	35.3
Old growth of main stem.....	0.0	36.7	0.0	23.5
Lateral branches.....	84.4	3.3	89.4	41.2
Number of observations.....	32	30	19	17
Mean tree height, in feet.....	3.6	3.6	6.3	6.3

The effect of *P. albicapitana* on the host tree depends on the location of the feeding larva. First-year larvae on vigorous terminals rarely cause damage. Second-year larvae cause only superficial injury to the tree when they feed on the older portions of the stem and branches. This damage is repaired by scar tissue after the adult emerges. If the second-year larva feeds at the base of a growing terminal shoot more serious damage results, as the shoot may be girdled and killed. Figure

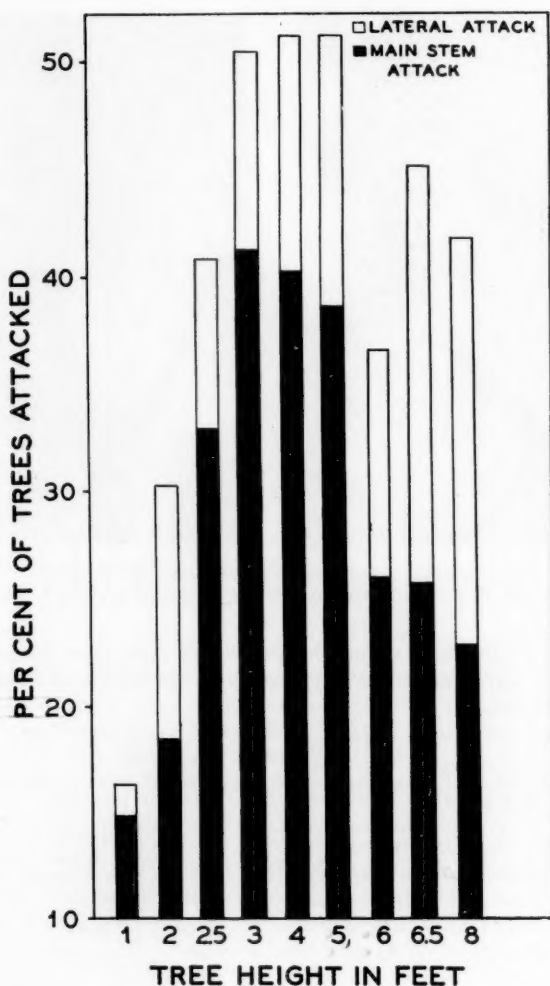


Fig. 6. Incidence of *P. albicapitana* on trees of different heights at Spruce Woods Forest Reserve, Manitoba.

4 shows a forked trunk caused by the killing of the leader by a second-year larva. More often, however, the deep wound causes the terminal shoot to grow in a crooked manner, and form a crooked and weakened trunk (Fig. 5.). This was the most common type of injury found in plantations. Breakage of the main stem of jack-pine saplings at the site of old nodule-maker injury was noted in some cases. The breaking occurred up to five years after the wound was made, and was probably caused by the additional stress of high winds and heavy snow. The less extreme distortions may be straightened gradually by growth but the weakness in the stem persists for a considerable time.

Factors Influencing Incidence and Damage

Susceptibility of Different Hosts

The host species, jack pine, lodgepole pine, and Scots pine, are not equally susceptible to attack by *P. albicapitana*. Jack pine in natural stands is more heavily and severely attacked than is lodgepole pine in natural stands. Neither red pine, *Pinus resinosa* Ait., nor white pine, *Pinus Strobus* L., were attacked by this insect in plantations or native stands.

In plantations where jack pine, lodgepole pine, and Scots pine are present in mixed or adjacent stands, the severity of attack on different hosts may be directly compared. Data gathered in Manitoba, Saskatchewan, and New Brunswick¹ show that lodgepole pine is much more susceptible to attack and damage in plantations outside its natural range than jack pine or Scots pine in adjacent plantations.

Age and Size of Host Trees

In the fall of 1948, insect rangers of the Forest Insect Laboratory, Winnipeg, conducted a survey of insects attacking plantations in the Spruce Woods Forest Reserve, Manitoba. From this survey the tree height and incidence of *P. albicapitana* were taken on 3,192 jack pine under 15 feet high in the Headquarters area. The pine were of mixed age and size and were dispersed among older pine plantations. Nodules of the 1949 brood infested 45.8 per cent of the jack pine under 15 feet high. Figure 6 shows the incidence of *P. albicapitana* in September, 1948, plotted against the tree height at the time eggs were laid in the spring of 1947.

Additional analysis of the effect of tree size on the incidence of *P. albicapitana* was greatly facilitated by records of the number of attacks by this insect on jack pine in experimental plots at Cloquet, Minnesota. These records were made available through the kindness of T. S. Hansen, Professor of Forestry at the University of Minnesota and Director of the Cloquet Forest Experiment Station, under whose

TABLE II
Incidence of *P. albicapitana* on Leaders and Laterals at Cloquet, Minnesota.

	Average number of nodules per plot		
	1946	1948	1950
Leaders.....	5.7	6.7	0.8
Laterals.....	15.7	34.3	—

direction the records were taken. The plots were planted in blocks of 25 trees between 1938 and 1940. The records show the incidence of nodules on the leaders and laterals of the trees in 49 blocks for the broods emerging in the years 1946 to 1949, and the mean height of the trees the fall before emergence. The record for the 1950 brood gives only the incidence on the leaders and the height the previous fall.

Table II expresses in terms of average number of nodules per plot the population changes of the broods emerging in even-numbered years. The smaller brood had such low populations, less than one nodule per plot, that no significant comparisons could be made.

¹Data provided by W. A. Reeks, Forest Insect Laboratory, Fredericton, New Brunswick.

TABLE III
Average Number of Nodules of *Petrova albicapitana* per plot on Jack Pine of Different Heights.

Height in feet	1948		1949		1950
	Leader	Total	Leader	Total	Leader
1-2	7	14			
2-3	11	21	1.0	1.0	
3-4	7	36	0.0	1.0	5.0
4-5	6	48	0.2	1.1	3.0
5-6			0.1	0.5	1.2
6-7			0.1	0.4	0.8
7-8					0.3
8-9					0.0

The population reached a peak in 1948 and then began to decline. The relation of these populations to tree height at time of oviposition is shown in Table III.

These data illustrate the build-up and decline of the population of *P. albicapitana* in relation to tree growth in jack-pine plantations. Trees under one foot high were not selected for oviposition by the adults. Trees between one and five feet high at the time of adult activity were increasingly suitable for oviposition. Attacks on the leaders were most abundant on trees between one and three feet high. Trees over five feet high were apparently decreasingly suitable for oviposition and the number of attacks, especially on the leaders, drops rapidly. On trees over 15 feet high incidence of *P. albicapitana* was low, although nodules were found on trees up to 50 feet high. On larger trees, the nodules were always located near the top where growth was vigorous, and never on suppressed branches or trees. Butcher (3) reported that nodules were more numerous on trees around the periphery of 25-tree plots than in the centre. It thus appears that exposed situations of the host may favour heavy attack by *P. albicapitana*, possibly due to chance encounter.

Isolation of Young Plantations as a Factor in Reducing Attack

In the Spruce Woods Forest Reserve, Manitoba, recent pine plantings have been confined to two areas. The first area is located around Headquarters, in Twp. 10, Rge. 16, WPM, and the other in Area A, lying about 4.5 miles north of Headquarters on both sides of Highway No. 1. The Headquarters plantations consist of 274 acres of pine planted between 1917 and 1930, and 286 acres planted since 1933. Area A has been developed since 1933 and contains 222 acres of pine plantations.

The 1948 plantation survey provided data by which the incidence of *P. albicapitana* on trees in the young plantations of the two areas might be compared. In Headquarters area, where the young plantations were among or adjacent to a source of adults from plantations 10 to 30 feet in height, 42.9 per cent of the young trees were attacked; in Area A, without a nearby source of infestation, only 19.4 per cent of the trees were attacked.

The incidence of *P. albicapitana* for four generations in two lodgepole pine plantations, one surrounded by older plantations and the other 750 feet northeast of the nearest source of infestation, was compared. There was no significant difference in the number of trees infested in these two plantations. The observations

indicate that the minimum effective distance for isolation of new plantations is less than 4.5 miles and more than 750 feet.

Natural Control

It seems probable that the most important natural control factor governing the build-up and maintenance of high population levels of *Petrova albicapitana* is the availability of suitable hosts for several successive generations. Other factors are responsible for considerable field mortality but their value in regulating the population is not known. Table IV presents some estimates of the amount of mortality that occurred among populations of *P. albicapitana* at Spruce Woods Forest Reserve for parts of the life cycle. These estimates were made from observations taken in different years and for two generations of the same brood.

TABLE IV
Mortality Observed Among Field Populations of *Petrova albicapitana* for
Different Parts of its Life Cycle, Spruce Woods Forest Reserve, Manitoba.

Part of life cycle	Period between	No. of observations	Per cent mortality
Overwintering larvae, early spring feeding, and migration	Oct. 1949— June 22, 1950.	19	10.5
Feeding second-year larvae	June 20, 1950— Nov. 10, 1950.	50	34.0
Overwintering larvae, early spring feeding, start of pupal period	Oct. 1947— June, 1948.	84	39.3

The cause of the mortality observed in the field could not be connected with specific factors in most cases. Parasitism of *P. albicapitana* will be discussed separately below. Other control factors that were noted in the field were bird predation of second- and third-year larvae, complete removal of the larva with the host tip by some browsing animal, and death of the larva caused by the drying of the host tissue after the branch was broken by the wind at the site of old pitch nodule maker injury. These factors are probably of minor importance. Physical control factors are probably responsible for much of the observed mortality, but climate does not appear to be a limiting factor in the distribution of *P. albicapitana* east of the Rocky Mountains.

Parasites

The degree of parasitism in populations of *Petrova albicapitana* was generally low, varying from 2 to 16.7 per cent in the material studied. Butcher and Hodson (3) regarded the low level of parasitism to be the result, in part, of the protected position of the larvae. Investigations have shown however, that the larvae are not protected at the time when adult parasites are active because they are migrating from their first-year nodule to a new location. The differences in parasite abundance appear to be correlated with the relative size of the host broods emerging in alternate years. The theoretical basis of this statement is that all evidence points to the parasites having a one-year life cycle, attacking the year-old migrating host larvae, and therefore attacking alternately the two host broods. If such is the case,

the parasite population would be limited by the size of the smaller of the two host broods. For example, at Cloquet where one brood has a very low population and the other is 49 times its size, parasitism was reported to be less than 2 per cent (3). At Spruce Woods Forest Reserve, where the broods are more nearly equal in size, one being only six times larger than the other, parasitism was reported to be 11.9 per cent. The populations of the dominant broods in the two areas were approximately equal.

Nine species of parasites were recovered from pupae of *Petrova albicapitana* that were brought into the laboratory. Additional information was obtained from the records of the Canadian Forest Insect Survey. The parasites recovered and their distribution are shown in Table V. Unless otherwise indicated, identifications were made by officers of the Systematic Unit, Division of Entomology, Ottawa. *Calliephialtes comstockii* and *Macrocentrus cuniculus* were most commonly recovered.

TABLE V
Parasites of *Petrova albicapitana*

HYMENOPTERA	
Ichneumonidae	
<i>Calliephialtes comstockii</i> (Cress.)	Quebec to Saskatchewan
<i>Campoplex</i> sp. nr. <i>sulcatellus</i> Vier.	Saskatchewan and Manitoba
<i>Scambus</i> sp. ²	Spruce Woods Forest Reserve, Manitoba
<i>Aphidius</i> sp. ¹	Hillsport, Ontario
Vipionidae	
<i>Apanteles petrovae</i> Walley	Minnesota, Ontario and New Brunswick
<i>Apanteles</i> sp. ¹	Merivale, Ontario
Braconidae	
<i>Macrocentrus cuniculus</i> Walley	Saskatchewan to Ontario
<i>Agathis</i> sp.	Spruce Woods Forest Reserve, Manitoba
Chalcidoidea	
<i>Hyssopus benefactor</i> (Cwfd.)	Brainerd, Minnesota
Tridymini	Spruce Woods Forest Reserve, Manitoba
<i>Hyssopus evetriae</i> (Gir.) ³	Cloquet, Minnesota
DIPTERA	
Tachinidae (det. H. J. Reinhard, College Station, Texas)	
<i>Lixophaga</i> sp. nr. <i>fasciata</i> Curran	Saskatchewan, Manitoba and Minnesota

¹ From records of the Forest Insect Survey.

² Possibly a hyperparasite.

³ From Butcher and Hodson. 1949.

Summary and Discussion

Petrova albicapitana (Busck) attacks jack pine and lodge pole pine throughout most of their natural ranges in North America. Plantations of these species and Scots pine are more heavily attacked than natural stands.

Two years are required to complete the life cycle. Two winters are passed in the larval stage; pupation and adult activity occur in the early summer. The young larva, protected by a small blister-like nodule, feeds on the cortex of the growing shoots. The nodule is composed of pitch, silk and frass. After passing the winter under a nodule the larva migrates to a crotch of the host tree and forms a new nodule. Under this nodule the remainder of the larval stage and the pupal stage is passed. The second-year larva feeds on the bark, cambium, and sapwood of the stem and damages the tree by killing, or distorting and weakening the leader of the main stem. Because of the two-year life cycle there are two broods, with adults emerging in alternate years. The size of these broods in a given locality is rarely equal.

In plantations, trees between one and five feet in height are most susceptible to attack and damage by this insect. Trees over eight feet high are rarely damaged.

Although nodules were found on jack pine fifty feet high, populations of this insect appear to depend on the presence of a succession of young susceptible hosts in large numbers. Isolation of new plantings from young plantations with *P. albicapitana* infestations is much more important than isolating them from mature pine stands.

Natural control factors, climatic and biotic, could not be shown to limit the populations of this insect under natural conditions. Only the growth of the host trees beyond the susceptible size was observed to reduce the insect population.

This investigation indicates that the following practices could be adopted to reduce damage should this insect become economically important in pine plantations. In the Prairie Provinces it is not generally feasible to plant resistant species, red pine and white pine. Preference in planting should be given to jack pine and Scots pine in that order. Lodgepole pine should not be used because of its susceptibility to attack. New plantations should be isolated from sources of infestation whenever possible. Afforestation plans should avoid the establishment of a succession of plantings in the same area. An area planted in one or two years will suffer much less damage than the same area planted in small blocks over a number of years. Nevertheless, this recommendation should not be adopted without consideration of other insect problems that might arise in even-aged stands.

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Notes on the Bionomics of Some Ontario Cercopids (Homoptera)¹

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When Manns (1939) reported that the meadow spittlebug, *Philaenus leucophthalmus* (L.), was a vector of peach yellows, the investigations on the bionomics of vectors of peach virus diseases then being carried on in the Niagara Peninsula were expanded to include this species. During the rearing of large numbers of nymphs to determine the host plants and habitats of *P. leucophthalmus* some other species of cercopids were also encountered.

Philaenus leucophthalmus (L.)

Prevalence. The meadow spittlebug is one of the most abundant insects in the Niagara Peninsula, and in many seasons its biomass undoubtedly exceeds that of any other species over large areas, especially in mixed farming districts. During the nymphal stages it is confined to situations with perennial or biennial herbaceous plants and with vegetation that provided suitable oviposition sites the previous season; but shortly after reaching maturity the adults disperse and are found in large numbers throughout the orchards of the fruit belt, even those a mile or more from extensive breeding grounds. The nymphs are generally absent and the adults scarce in dense woods, although the latter may occur in the tree-tops as they are often abundant on the terminal twigs of large apple trees.

No detailed population counts were made, but at Vineland Station more than 500 nymphs were collected from about 10 sq. ft. of an alfalfa field and approximately the same number from about 25 sq. ft. on a weedy hillside in both 1942 and 1943.

A spectacular concentration of the adults appeared in early July, 1945, along the Lake Ontario shore. They were first noticed about 6.00 p.m. on July 5; the temperature was about 85°F. with a slight southwest breeze. Attention was drawn to them by the patter, like rain, as they hit the leaves of trees. They were flying in a northeasterly direction at all levels from just above the ground to as high as they could be seen. As they reached the lake they tended to fly eastward parallel to the shore and eventually lighted on any nearby vegetation. The stems of plants in the immediate vicinity of the shore were often covered with solid masses of the hoppers; more than 70 were counted on a single head of rye. Beneath heavily infested trees the excreta could be felt falling as a mist that wet the plants beneath. The greatest concentration occurred within 5 to 10 yd. of the shore but the hoppers were also very abundant as far as a quarter of a mile away. The insects were personally seen to be more or less uniformly distributed for about a mile both east and west of Vineland Station, and according to other reports the concentration extended for 20 mi. or more along the lake. The hoppers occurred in greatest numbers for seven or eight days and then rapidly dispersed or died until relatively few were left after two weeks.

As the nymphs had not been noticed in unusual numbers in the vicinity of Vineland Station, and no reports of injury to strawberries or other crops had been received, it was apparent that the adults had flown from a long distance. During the few days previous, a spell of good haying weather after a period of rain had allowed a very large acreage of meadow to be mowed in the mixed farming area south of the Niagara Escarpment, forcing a movement of the insects, which drifted toward the lake on a warm wind.

¹Contribution No. 3014, Division of Entomology, Science Service, Department of Agriculture, Ottawa, Canada.

²Agricultural Research Officer.

Life-History. Nymphal development was followed by examining nymphs collected at approximately weekly intervals. In 1942 they were collected from an alfalfa field about 200 yd. from the lake, designated Plot 1. As development appeared to be somewhat slower in this location, because of the cooling influence of the lake, in 1943 collections were made both in Plot 1 and on a weedy south slope, Plot 2, about 600 yd. from the shore. The percentage of each instar are given in Table I.

TABLE I
Development of the Meadow Spittlebug
at Vineland Station, Ont.

Date	Total nymphs	Percentage of each instar					
		1st	2nd	3rd	4th	5th	Adults
<i>1942 — Plot 1</i>							
April 30	30	77	23				
May 6	84	6	94				
May 13	117	4	67	29			
May 21	101		11	74	15		
May 27	136		2	46	52		
June 3	136		1	8	71	20	
June 10	156			1	9	90	Few
June 17	114				5	95	Many
(Alfalfa cut about June 20)							
<i>1943 — Plot 1</i>							
May 13	Not hatched						
May 24	91	52	48				
May 31	127	9	57	34			
June 9	147		6	14	79	1	
June 14	126		2	4	47	47	None
June 21	114				4	96	Many
<i>1943 — Plot 2</i>							
May 13	30	60	30				
May 24	102	5	40	53	2		
May 31	122	1	5	38	56		
June 9	103				39	61	
June 14	104		1	1	4	94	Few
June 21	All matured						Many

In 1942 hatching of the eggs was nearly or quite complete at the first examination of Plot 1 on April 30. In 1943, a later season, hatching commenced in Plot 1 very shortly after May 13; in Plot 2 the majority of the eggs had hatched by that date.

The first adults in 1941 were collected by sweeping on June 9. In 1942 the first matured in Plot 1 a day or two before June 10; in 1943 they began to mature in Plot 1 between June 14 and 21, and in Plot 2 between June 9 and 14. In the latter year maturity was complete in Plot 2 by June 21, and about a week later in Plot 1.

The adults remain abundant throughout July and August; by September their numbers are reduced but some survive until severe frost in late October or early November.

Hosts and Feeding Habits. Doering (1942) and DeLong and Severin (1950) have published long lists of plants on which the meadow spittlebug has been found, although they do not always distinguish between feeding or resting hosts of the adults and breeding hosts on which the nymphs develop. The writer found the nymphs feeding, as evidenced by production of froth, on at least 91 species of plants belonging to 29 families. The majority were dicotyledonous perennial or biennial herbs; few annuals are large enough to be infested at the time the nymphs hatch but the latter often move to them later. Where the nymphs were abundant it was the usual experience to find them on all dicotyledonous herbs of sufficient size, a notable exception being common mildweed, *Asclepias syriaca* L., which was never attacked even when closely surrounding vegetation was heavily infested.

Hosts other than dicotyledons were *Equisetum arvense* L., *Alisma triviale* Pursh, and *Scirpus* sp. A single nymph was found in a mass of froth on quack grass, *Agropyron repens* (L.) Beauv., probably temporarily established as grasses were normally avoided. Nymphs were occasionally found on succulent shoots of woody plants, including apple, pear, sweet cherry, *Cornus racemosa* Lam., and *Sambucus canadensis* L.

Plants on which the adults were found included most of the common wild and cultivated local flora, but which were actually fed upon was seldom known. Some adults lived at least two months in cages on peach trees, and they apparently fed freely on the foliage and young twigs of other trees as well as herbs.

The effect of nymphal feeding upon a plant varied with the point of attack; this was particularly noticeable in a plot of garden chrysanthemums. Nymphs established on the lower part of the stems below the zone of elongation, or on the midribs of fully expanded leaves, did not produce any obvious effects, although growth may have been reduced when many nymphs were present. On elongating parts of the stems they often caused curvature and dwarfing, and on expanding leaves they produced curling. Attack near the tip of the shoot produced the most severe injury, the plant sometimes being reduced to a low rosette of distorted leaves. Similar responses were noticed on other hosts.

Ordinarily the feeding of the adults did not cause any noticeable injury. Massive attack by the unprecedented concentration in July, 1945, mentioned previously, killed buckwheat and other succulent plants, and much of the foliage on more mature herbs wilted and died. The leaves on peach nursery stock wilted badly; the stock appeared to recover after the insects had been destroyed with DDT but it made little or no subsequent growth. Strings of gum exuded from some peach fruits on which large numbers of hoppers had fed.

When 10 or more adult spittlebugs were caged on peach seedlings about 18 in. high, the tips of the shoots and sometimes the entire plant wilted and frequently died.

A few feeding punctures of adults of the meadow spittlebug on peach twigs were sectioned incidental to a study of the feeding habits of some cicadellids. Although much larger in diameter, the punctures were generally similar to those of *Macropsis* spp. (Putman, 1941), penetrating intracellularly to the vicinity of the cambium. Some punctures ended in the phloem and others extended into the outer xylem; the number examined was not enough to show what tissues were most usually fed upon. Copious deeply staining sheath material was deposited along the passage made by the stylets.

Oviposition. Older writers stated that the eggs of *Philaenus leucophthalmus* were inserted within the stems of plants, probably an assumption based on the

presence of a well-developed ovipositor. Barber and Ellis (1922) found the eggs inserted between the stems and leaf sheaths of grasses and embedded in a white frothy material, and Mundinger (1946) found them in similar situations. Ahmed and Davidson (1950), who have given the most complete account of the life-history, found the eggs on alfalfa, inserted between the stems and sheaths or in cracks on the stems of stubble. Weaver (1951) said that first-year legume meadows were most severely infested because most of the eggs were laid on the grain stubble the preceding year; older meadows seldom bore economically heavy infestations.

The writer found the eggs on alfalfa stems, cemented between the stems and stipules, as described by Ahmed and Davidson, and local alfalfa stands three or more years old contained exceedingly large populations of nymphs, practically every stem bearing one or more.

Colour Varieties. Several of the named colour varieties of this species, as well as many intermediate forms, were present in any lot of reared or collected adults. There was no apparent relation between these varieties and hosts, habitat, or other aspect of the bionomics of the species.

Production of Spittle. Several authors have described the process of froth or spittle formation, but the source of the liquid has been in dispute. Batelli (1918) described glands on the seventh and eighth abdominal segments that secrete a semi-solid material which is mixed with the excretion from the anus. Garman (1921) also described glands in the same location in *Philaenus lineatus*, which he stated supply a large part of the fluid. Cecil (1930), however, could not find these glands in *P. leucophthalmus* and said that the liquid was produced entirely from the anus.

The writer noticed that, when nymphs of any of the species mentioned in this paper were immersed in 70 per cent alcohol, a mucus-like material coagulated laterally on the seventh and eighth abdominal tergites, presumably produced by the glands of Batelli. In *Aphrophora signoreti* these secreting areas are white in strong contrast with the red of the rest of the terga. At least the greater part of the liquid forming the spittle must be emitted from the anus but it flows forward over the tergal secretion and may mix with it.

Aphrophora signoreti Fitch

According to Ball (1934) and Doering (1941), *Aphrophora signoreti* Fitch is an uncommon species with its centre of distribution in Ontario. The nymphs were recorded from grape and the adults from pine by these authors. In the vicinity of Vineland Station the nymphs were found on low vegetation in mixed woodland with scattered white pines; in a small plantation of young Scots pine; and beneath some small trees of white spruce. They were not found beyond about 20 ft. from the tall white pines and were mostly beneath the spread of the branches of the smaller conifers. Host plants were *Quercus rubra* L. (seedlings), *Actaea* sp., *Barbarea vulgaris* R.Br., *Ribes americanum* Mill., *Geum aleppicum* Jacq. var. *strictum* (Ait.) Fern., *Rubus odoratus* L., *Geranium maculatum* L., *Oenothera biennis* L., *Circaea quadrisulcata* (Maxim.) Franch. & Sav., *Lysimachia nummularia* L., *Fraxinus americana* L. (seedlings), *Collinsonia canadensis* L., *Galium triflorum* Michx., *Viburnum acerifolium* L., *Solidago caesia* L., *S. flexicaulis* L., *S. altissima* L., *Aster macrophyllus* L., *Arctium minus* (Hill) Bernh., and *Prenanthes* sp. Nymphs from several of these hosts were caged on *Solidago altissima*; practically all reached maturity and were determined as *A. signoreti* by G. S. Walley, Division of Entomology, Ottawa.

The nymphs, in the third to fifth instars, were found during June, 1941, and reached maturity during the last week of the month. The adults disappeared

from the vegetation on which the nymphs had fed immediately after they had matured.

The life-history of *Aphrophora signoreti* is probably similar to that of *A. saratogensis* (Fitch), as described by Secrest (1944) and Anderson (1947), the nymphs feeding on various herbs and shrubs and the adults on conifers, especially pines. Secrest said that the eggs of *A. saratogensis* were inserted in the stems of the hosts of the nymphs, whereas Anderson claimed they were laid in the sheaths of the needles and under the bud scales of pine. The fact that the nymphs of *A. signoreti* were never found farther than a few feet beyond the spread of the branches of pines and spruces suggests that the eggs were laid on them.

Only the last three nymphal instars were found. These are similar to the corresponding stages of *A. saratogensis* as described by Anderson. In the third and fourth instars the abdomen is bright scarlet and the head, thorax, and legs dark fuscous to black with some paler markings. In the fifth instar the abdomen varies from brownish-yellow to pink; the other parts are more brownish than in the earlier instars and the lighter areas more extensive and variable.

Other Species

Philaenus lineatus (L.) was the commonest local species next to *P. leucophthalmus*. The nymphs were found only on the grasses *Agropyron repens* (L.) Beauv., *Arrhenatherum elatius* (L.) Mert. & Koch, *Festuca elatior* L., *Phleum pratense* L., *Poa compressa* L., and *P. pratensis* L. Both nymphs and adults of *Aphrophora parallela* (Say) were very abundant on young Scots pine. Nymphs of *Clastoptera proteus* Fitch were rather common on *Cornus stolonifera* Michx., *C. alba* L., and *Cornus* sp. (unidentified ornamental). A few nymphs of *Clastoptera obtusa* Say were found and reared on *Carpinus caroliniana* Walt.

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Observations on Hyperparasitism of the Wheat Stem Sawfly *Cephus cinctus* Nort. (Hymenoptera: Cephidae)¹

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The biology of *Bracon cephi* (Gah.) (Hymenoptera: Braconidae) was described recently (Nelson and Farstad, 1953). During studies from the Field Crop Insect Laboratory, Lethbridge, on the biology of this important parasite, observations were made on various other hymenopterous parasites associated with the wheat stem sawfly, *Cephus cinctus* Nort. The parasites discussed herein are *Eupelmella vesicularis* (Retz.) (Eupelmidae), *Eurytoma atripes* Gah. (Eurytomidae), and *Merisus febriculosus* Gir. (Pteromalidae). These three parasites have been noted by Gahan (1933) as parasites of the hessian fly, *Phytophaga destructor* (Say).

Pleurotropis utabensis Cwfd., taken only as a primary parasite during these studies, was discussed by Neilson (1949). *Eupelmus allynii* (French), recorded from *C. cinctus* and from *B. cephi* by Criddle (1924), was not taken by the present author.

Eupelmella vesicularis (Retz.)

The biology of *E. vesicularis* as a parasite of the hessian fly was well described by McConnell (1918). As a parasite of jointworms (*Harmolita* spp.) its biology was described by Phillips and Poos (1927). The insect was discussed by these authors under the name *Eupelminus saltator* Lindemann. It has been recorded from *C. cinctus* by Ainslie (Gahan, 1933) at Bottineau, North Dakota.

The habits of the adult are as described by the earlier authors. Oviposition on larvae of *C. cinctus* and *B. cephi* within wheat stems was observed in experimental containers. Only under these experimental conditions, when several eggs were laid near the one host, did the author observe the net-like structure observed by Phillips and Poos (1927), by means of which eggs are fastened to the inside wall of the host hibernaculum. The insect overwinters within the stem in the larval stage, and the adult emerges through a small circular hole cut in the stem wall. The life-span of the adult insects is not known, but Phillips and Poos (1927) showed that they lived an average of 26 days under favourable conditions.

Adults are very difficult to take with a net in a field of growing wheat. Once the wheat has been harvested, however, they are easy to take by sweeping. As the insects are wingless, they are found mostly on the lower parts of the wheat plant.

The life-cycle of *E. vesicularis* has become well synchronized with those of its hosts in the Canadian prairies. Immature stages may be found in early spring, both free within wheat stems (indicating *C. cinctus* as host), and within *B. cephi* cocoons. The adult emerges during the first two weeks of May and attacks overwintered larvae of *B. cephi*, and in early June attacks pupae of *B. cephi*. Second-generation females emerge at the same time as first-generation adults of *B. cephi*, during the last part of June and early July. These females, together with those of *B. cephi*, attack *C. cinctus* larvae, beginning about mid-July. During early August, larvae and free pupae are found in wheat stems, and third-generation adults emerge about the fifteenth.

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Whether or not the third generation attacks *C. cinctus* and *B. cephi*, or *B. cephi* alone, depends on the weather. In dry weather, *C. cinctus* larvae are unavailable for parasitism (Nelson and Farstad, in preparation), and *B. cephi* larvae are the only hosts available, as they may be present at any point in the wheat stem. In a wet season, however, *C. cinctus* larvae reach the bases of the stems later, and remain available. It was not established whether a fourth generation occurs; it is possible that in ideal September weather a fourth generation emerges. If so, hosts are automatically overwintering *B. cephi* larvae.

Eurytoma atripes Gahan

When *Eurytoma atripes* was described by Gahan (1933), 22 specimens had been reared from *Phytophaga destructor* by various workers, and one specimen from *C. cinctus* by Ainslee in North Dakota.

The habits of this insect as a parasite of *C. cinctus* correspond closely with what Gahan has already noted about it as a parasite of *P. destructor*. It was found, however, to be both a primary and a secondary solitary parasite feeding externally on larvae of *C. cinctus* and on larvae and pupae of *B. cephi*. Activity of the adult is very difficult to follow. Oviposition was not observed, either in the field or under artificial conditions. The adults do not fly swiftly, but their black colour and small size make them difficult to see in flight. They are easier to take with a net from stubble than from uncut grain. Eggs are similar to those of *Eupelmella vesicularis*, as they have both a pedicel and the appendage termed a *flagellum* by McConnell (1918, pp. 171-2).

E. atripes is often associated with *Eupelmella vesicularis* in attacking *C. cinctus* and *B. cephi*, and the species are often present in equal numbers. Its life-history appears to be identical with that of *E. vesicularis*, and it appears to be subject to the same hazards of availability of hosts in the fall of the year.

Merisus febriculosus Girault

One male of *Merisus febriculosus* was reared by the author from a cocoon of *B. cephi*. One female was observed ovipositing in a wheat stem in the field on September 10, 1946, at Aylesbury, Saskatchewan. Dissection of the stem showed that the intended host was *B. cephi* but that no egg had been laid.

Economic Importance

E. vesicularis and *Eurytoma atripes* apparently occur throughout the wheat stem sawfly area of Western Canada, as they have been taken from various points in Alberta and Saskatchewan. They are of little economic importance as parasites of *C. cinctus*. At Aylesbury, Saskatchewan, in May, 1946, a total of 7.6 per cent parasitism of *C. cinctus* by these parasites was noted in overwintered wheat stubble. In June of the same year, however, at least 20 per cent parasitism of *B. cephi* larvae and pupae was noted. The Aylesbury district is the only locality in which the parasites have been found in economic numbers. It is questionable whether, under such conditions, these parasites materially influence parasitism of *C. cinctus* by *B. cephi*.

Summary

The life-histories of *Eupelmella vesicularis* (Retz.) (Hymenoptera: Eupelmidae) and of *Eurytoma atripes* Gah. (Eurytomidae) as parasites of the wheat stem sawfly, *Cephus cinctus* Nort., are outlined. Adults of both species emerge in May and attack overwintering larvae and pupae of *Bracon cephi* (Gah.). Second-generation adults emerge at the same time as *B. cephi* adults in June and attack *C. cinctus* larvae. Third-generation adults emerge in mid-August and may

attack both *C. cinctus* and *B. cephi*. They are of little economic importance. *Merisus febriculosus* Gir. (Pteromalidae) is recorded from *B. cephi* and is of no economic importance.

Acknowledgments

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A Species of *Tetrastichus* New to North America

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During studies of the parasite complex of the diamondback moth, *Plutella maculipennis* Curt., at Ottawa, Ontario, July to October, 1952, and April, 1953, 1,234 host cocoons were collected from a heavily infested field of Penn State Ballhead cabbage. On October 31, eight adults (2 ♂ and 6 ♀) of *Tetrastichus sokolowskii* Kurdj., emerged from a single cocoon collected 16 days previously and held under laboratory conditions in a gelatin capsule. On May 10, nine adults (1 ♂ and 8 ♀) and on May 15, eight adults (1 ♂ and 7 ♀) of the same species emerged from two overwintered cocoons collected 14 and 16 days previously. In each case, the lack of parasitic remnants indicated that they were primary parasites.

The species has not been previously recorded from the Nearctic region (Peck, 1951). The determination was made by O. Peck of the Systematic Entomology Unit, Division of Entomology, Ottawa, and confirmed by B. D. Burks of the Bureau of Entomology and Plant Quarantine, Washington. The former (in litt.) states that *T. sokolowskii* was originally reared from the diamondback moth in Russia and has since been recorded only from southern India.

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Lipolytic Enzymes Extracted from *Galleria mellonella* L. (Lepidoptera:Pyralidae) Reared on Natural and Artificial Media

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While rearing *Galleria mellonella* L. to further studies by Mankiewicz (1952) on the wax splitting enzymes which they produce and the action of these enzymes on *Myctobacterium tuberculosis*, it proved difficult to secure a constant supply of honey and comb, the natural food and habitat of this species. Haydak's (1936) summary of earlier work on rearing this wax moth indicated that success had been attained with several artificial media. The writers, therefore, tried an artificial medium and attempted to assess its effect on the nature of the enzymes later extracted from the larvae.

Experimental Procedure

The artificial rearing medium consisted of:

Fine corn meal.....	20 grams
Whole wheat flour.....	40 grams
Skim milk powder.....	20 grams
Powdered dry yeast.....	10 grams

These materials were mixed dry and then stirred up with equal amounts of liquid honey and glycerine until the mixture had the consistency of wet sand. Two inches of the material were placed in the bottom of each screen covered candy jar and seven hundred eggs, previously collected by having mated female adults lay on crumpled wax paper, were added. Four weeks later large healthy looking larvae, mostly in the last instar, were collected from the cultures and frozen until the enzyme extracts could be prepared. These extracts (Mankiewicz 1929 and 1952) were prepared from ground larvae therefore contained both cellular and digestive enzymes. Several thousand larvae were collected separately from the artificial medium and from cultures reared on honey and comb in similar jars.

Results

1. The thawed larvae from the artificial medium were more uniform yellow in color while those from the honey and comb cultures contained many black and brown larvae. Similarly the faecal matter present in the artificial culture larvae was yellow rather than black.

2. When thawed larvae were ground up in a Waring Blendor the color difference remained. The blend of larvae from honey and comb was dark, sticky, oily, and blackened where it contacted the Blendor blades while the other sample was light yellow, fine, homogenous and did not blacken on contact with the metal.

3. It required 3,350 ml. of acetone to extract the fats from 75 grams of the honey and comb reared blend until the acetone came off light yellow while 520 ml. did the same job for the blend of larvae reared on the artificial medium.

4. Extracts of the total enzymes in the defatted blends were prepared from one gram of larval powder to 8 ml. of suitably buffered solution (Mankiewicz 1952) and different lipid substrata subjected to their action for 24 hours at 37 degrees C. The free fatty acids thus liberated were titrated with 0.1 N KOH using phenolphthalein as an indicator. All tests were in triplicate and included controls of heat inactivated enzyme extract. The average number of millilitres of 0.1 N KOH used to neutralize the acids in each instance after 100 ml. of extract had acted for 24 hours were:

For a substratum of:	<i>Tributylin</i>	<i>Olive Oil</i>	<i>Ethyl-n-Butyrate</i>	<i>Beeswax</i>
and the extract from comb fed larvae	1,400	500	1,600	500
the extract from artificial medium larvae	900	500	1,100	350

Discussion and Conclusions

The enzymes present in the extracts are a mixture of all the enzymes in the larvae not just those in the gut, but one cannot avoid the conclusion that the different diet altered this enzyme complex in some way. That such an effect might well alter the susceptibility of the larvae to different toxins seems highly probable. No tests were conducted on susceptibility but it is hoped to carry out such tests later. Both Swingle (1922) and Markos & Campbell (1943) have reported dietary effects on insecticide susceptibility to arsenic and it is generally recognized that the host plant influences the ease with which certain insects are controlled.

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Varietal Responses of Seeded Onions to the Onion Maggot

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From 1945 to 1949 tests were conducted at Ottawa to determine whether certain of the more commonly grown commercial varieties of onions are resistant to the onion maggot, *Hylemya antiqua* (Meig.). Sleesman (1934), from the results of field tests in Ohio, had concluded that some varieties of onions do support significantly higher populations of the maggot than others.

In 1945 and 1946 at Ottawa, 17 varieties of onions were tested; in 1947, 1948, and 1949 the number was reduced to nine, which are listed in Table I.

All of the tests were conducted in the same experimental field, the soil of which was a uniform sandy loam. In 1945 and 1946 the individual plots consisted of three rows 20 feet in length, replicated five times in a randomized design. In the other three years they consisted of two 30-foot rows replicated five times in randomized blocks. The seed was planted by hand in early May at one-fifth of an ounce per 60 feet of row from 1945 to 1948, and at rates varying with the variety in 1949. The criterion of varietal resistance was the percentage of plants killed by the maggot from early June until mid-July. Seedling mortality counts

were made twice each week in June, and at weekly intervals in July. By mid-July the plants were large enough to withstand the attack of the maggot.

TABLE I
Percentages of Seeded Onion Plants Killed By The Onion Maggot
In June And July, 1945 To 1949, Ottawa

Onion variety	Number of plants examined					Per cent killed					
	1945	1946	1947	1948	1949	1945	1946	1947	1948	1949	Mean
Red Wethersfield	3752	2272	2520	674	2701	4.4	25.8	8.3	2.1	15.5	11.2
Brown Australian	2561	2453	4413	2787	2393	7.3	18.2	8.4	8.0	15.7	11.5
Ailsa Craig	3570	2904	4587	3409	2960	5.0	23.0	13.1	7.9	13.7	12.5
Sweet Spanish Utah	2935	2986	5450	3702	2383	4.6	24.4	7.0	9.2	17.8	12.6
Yellow Globe Danvers	2656	1489	1529	3561	2546	7.9	26.5	4.6	11.7	15.3	13.2
Sweet Spanish Riverside	3609	2858	3923	3505	3256	4.8	28.0	15.2	10.2	17.3	15.1
White Portugal	4025	3734	6656	4475	3417	5.3	37.1	10.4	10.6	14.7	15.6
Ebenezer	4061	3280	4416	3235	2171	7.1	31.4	16.7	12.1	12.2	15.9
Early Yellow Globe	2643	2255	4086	2795	1572	4.7	37.3	20.7	10.8	9.2	16.5
Mean	3312	2692	4175	3127	2600	5.7	28.0	11.6	9.2	14.6	13.7

Table I shows that there were varietal differences in plant mortality caused by the maggot but that these differences were not consistent. In 1945, the infestation was too slight to indicate significant differences between varieties; in three of the other four years, 1946 to 1948, Red Wethersfield and Brown Australian were slightly more resistant than Ebenezer and Early Yellow Globe, but not in 1949.

Of the eight varieties tested by Slesman, in one year only, Ebenezer had the highest maggot population whereas Early Yellow Globe was intermediate.

At Ottawa, the infestation was high in 1948, moderate in 1947, and low in the other three years. The experiments suggest that there are differences in the resistance of certain varieties of onions to the onion maggot, but that on the average the differences are too slight to be significant in the low infestations that usually occur at Ottawa. When infestations are severe the differences may be obscured by variables resulting from uneven plant establishment. Table I shows that the number of plants established varied considerably between varieties from year to year, and these differences may have influenced the results. Seed germination tests were conducted in 1949 to try to reduce this variable, and the attempt was partly successful. In further tests of this nature, allowances should be made as well for varietal differences in the size of the seed.

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Observations on Internal Parasites (Hymenoptera: Scelionidae) of Eggs of Pest Grasshopper Species in the Prairie Provinces of Canada¹

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Introduction

Internal parasites belonging to the genus *Scelio* Latr. have been reported found in grasshopper eggs in several of the northern states of the United States. Muesebeck and Walkley (1951, p. 702) reported *S. calopteni* Riley in *Melanoplus* spp., including *M. mexicanus mexicanus* (Sauss.) and *M. bivittatus* (Say), in states adjacent to the Prairie Provinces of Canada. In Canada, Criddle (1921) reported *Scelio* sp(p). in Manitoba during a grasshopper outbreak which took place about 1920. Since then, the presence of *Scelio* sp(p). in grasshopper eggs has frequently been noted in the Prairie Provinces by entomologists. Studies of the viability, embryonic development, and parasitism of Western Canadian grasshopper eggs begun and largely carried out by the late Mr. H. W. Moore at the Field Crop Insect laboratories at Brandon and Saskatoon have made possible an approach to the quantitative evaluation of these parasites.

Although Scelionidae have probably seldom, if ever, been of critical importance in Western Canada, they now appear, from information on record, to be of greater importance than previously suspected. They clearly make a worthwhile addition to the effects of the several known egg predators, the numerous known internal parasites of the nymphal and adult stages, and the many arthropod, avian, and mammalian predators. Unimpressive singly, these natural control factors when combined are worth a great deal to Canadian agriculture.

At present, the taxonomy of *Scelio* spp. in North America is said to be in need of revision. Locality and host records of *Scelio calopteni* Riley from some northern states of the U.S.A. suggest that it should be represented in Western Canada (cf. Muesebeck and Walkley, p. 702). Treherne and Buckell (1924) stated that Mr. N. Criddle, Entomological Branch, informed them that *S. calopteni* had been reared from *Melanoplus* eggs in Manitoba. Adult parasites reared from eggs of *M. m. mexicanus* and *Cammula pellucida* (Scudd.) are at hand, but none have yet been obtained by the author from *M. bivittatus*. Therefore, it is uncertain whether one or several species are involved, and whether there is host specificity.

Methods

Methods of collecting and preserving the eggs examined during this investigation were described by Moore (1948). Most of the eggs were collected late in the fall, after soil temperatures favourable to the incubation of eggs had ended but before the onset of winter with its four to five months of freeze-up, and before any diapause or diapause potential in either grasshopper or parasite embryos had been broken. The eggs were preserved either in 70 per cent ethyl alcohol or by cool temperatures. Prior to examination, the eggs, whether in alcohol or alive, were cleared by placing them in turpentine (*Melanoplus* spp.) or benzene (*C. pellucida*) for eight to 10 days. They were then examined microscopically, usually under magnification of 16 diameters. In newly collected grasshopper eggs, there is a chance that some eggs will have been so recently parasitized that the parasites are not observed. Parasite eggs were not observed, and it is not certain whether

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this was due to failure to detect them, or whether all eggs had hatched prior to processing.

Biology

Most of the parasites observed in this study were seen in the bulbous, long-tailed first larval stage described from several authors by Clausen (1940). Moore (1945) described *Scelio* larvae as observed in whole grasshopper eggs during the present investigations as follows: "The parasite [first-instar larva] appears as a clear bubble with a curved tail when the egg is cleared, generally near the centre or at the anterior end of the egg." In a very few cases, second- and third (last-) stage larvae have been recognized; some pupae were found, but these were a small minority. In a few cases, pupae and first-stage larvae were found in eggs from the same egg-pod. Birch (1945) also found these two stages together in the same collections of eggs of *Austroicetes cruciata* Sauss. parasitized by *Scelio chortoicetes* Frogg.

Criddle (1921) stated that *Scelio* sp(p). require a full year to mature, and that they emerge when the host grasshoppers are laying eggs. In laboratory incubation and hatching of field-collected grasshopper eggs, the author has commonly found *Scelio* adult parasites emerging. Under laboratory conditions at a constant temperature of about 80°F., parasites began to emerge from batches of grasshopper eggs within one to two weeks after hatching of the last grasshopper nymph. Under field conditions it would therefore be expected that the period between the end of hatching of grasshopper eggs and the beginning of emergence of adult parasites would be at least double that time. It should be noted that under natural conditions the hatching period of economic grasshoppers often extends to the end of June, a month or more after the beginning of hatching by the same species. If the order of events under natural conditions is the same as that in the laboratory, some emergence of parasite adults would in fact be expected about the time oviposition by the host species begins. Initial emergence of scelionid parasites and hatching of grasshopper nymphs from an egg-bed at the same time, as observed by Noble (1935) for *Scelio fulgidus* Cwfd. from eggs of the Australian plague grasshopper, *Chortoicetes terminifera* (Walker), has not been observed and seems unlikely in Western Canada.

Oviposition habits of *Scelio* sp(p). have not been directly observed by the author or any of his associates. It has been noted that, if one egg in a pod contains a parasite, either most or all of the eggs in that pod are affected. Up to seven larvae in a single host egg have been seen in rare cases. Noble (1931) observed that where this happens one survivor destroys the rest. Moore (1945) has recorded that, in the present work, no trace of a grasshopper embryo had been observed in a parasitized egg. The indication is, therefore, that oviposition by the female parasite occurs at an early stage in the life of the host egg. In future investigations, observers may expect a situation similar to those reported by Clausen (1940) and Uvarov (1928, pp. 113-116), or Noble (1938). The former reported, from the results of workers in various parts of the world, that a female parasite may attend the ovipositing grasshopper closely and proceed to parasitize the grasshopper eggs immediately after or even before completion of oviposition by the host. Noble found most of the parasite oviposition within 24 hours of host egg-laying. Phoresy, reported by Clausen and by Uvarov from the work of several authors, was not observed by the present author or his associates. Because numerous large grasshopper collections have been handled over a period of years, it is unlikely that observations of phoresy would have been entirely missed if it occurs in Western Canada.

Prevalence

Some data on egg parasitism in Western Canada are available since 1941; the results to 1944 were reported by Moore (1945), who stated that the incidence of parasitism was usually less than 5 per cent, sometimes 10 to 15 per cent, and, more rarely, 20 to 30 per cent in egg collections of *M. m. mexicanus* and *M. bivittatus*. Moore stated that parasites were more prevalent in *M. bivittatus* than in *M. m. mexicanus*, and least prevalent in *C. pellucida*. Such results are typical of the more comprehensive data collected since 1944 (Table I).

TABLE I

Scelionid Parasitism in Eggs of *Melanoplus m. mexicanus*, *M. bivittatus*, and *Camnula pellucida* in the Prairie Provinces of Canada, 1945-51

Province	Year	Source and Constitution of Sample	No. of eggs	Per cent parasitized
<i>Melanoplus m. mexicanus</i>				
MANITOBA	1948	southwest, several points	602	0
	1950	Red River Valley, several points	895	2
SASKATCHEWAN		southwest, several points	771	3
	1945	Shaunavon, single field ¹	4,976	4
	1946	southwest, several points	523	2
		Kinley, single field	755	tr
	1947	general, three points	1,612	0
	1948	central, three points	2,302	1
	1949	general, several points	3,091	8
	1950	Davidson, single field ¹	529	9
	1951	Regina Plains, several points	145	0
ALBERTA		southwest, several points	271	1
	1946	Milo, single field ¹	3,129	25
	1949	Lomond, single field ¹	13,383	7
<i>M. bivittatus</i>				
MANITOBA	1948	Red River Valley, several points	2,725	0
	1950	Red River Valley, several points	2,118	3
SASKATCHEWAN		southwest, several points	1,960	6
	1951	Red River Valley, several points	2,527	24
	1946	southwest, several points	846	0
	1947	general, several points	4,812	3
	1948	south-central, several points	3,301	3
	1949	general, several points	2,791	14
	1951	Regina Plains, several points	873	3
		southwest, several points	658	3
ALBERTA	1949	Lomond, single field ¹	1,838	3
	1950	Carmangay, several points	1,770	26
		Hussar district, single point	1,430	0
<i>Camnula pellucida</i>				
MANITOBA	1948	Red River Valley, several points	955	0
	1950	Red River Valley, several points	868	0
SASKATCHEWAN		southwest, several points	909	0
	1951	Red River Valley, several points	765	10
	1946	Herschel, single point	993	0
		southwest, two points	2,035	0
	1947	general, five points	3,501	0
	1948	general, three points	3,078	1
	1949	general, several points	3,562	0
	1950	Davidson, single field ¹	559	0
	1951	Regina Plains, several points	143	0
ALBERTA		southwest, several points	152	0
	1950	Carmangay, several points	1,083	0
		Drumheller, several points	1,216	0

¹Representative collections from tillage experimental sites.

Parasitism of Various Species in Relation to Host Abundance

Among about 33,000 eggs of *M. m. mexicanus* examined during the period 1945-51, only a few collections showed considerable percentage parasitism by *Scelio* sp(p). For example, in Saskatchewan in 1949, the maximum parasitism in a single collection was 19 per cent, contributing substantially to the average figure of 8 per cent for the general area (Table I). This was the third consecutive year of grasshopper egg abundance in this province. In Alberta, eggs of *M. m. mexicanus* collected from a tillage experimental site in 1946 contained an unusually heavy infestation of *Scelio* sp(p), 25 per cent. This was at the end of several consecutive years of abundance of *M. m. mexicanus* in the locality.

In the host species *M. bivittatus*, the incidence of parasitism was roughly similar to that noted in *M. m. mexicanus*. *M. bivittatus* is an important species in the Red River Valley of Manitoba, and had been abundant there for several consecutive years before the autumn of 1951, when several collections of eggs were heavily parasitized; for example, a collection from Oak Bluff was 40 per cent parasitized. In Saskatchewan, there was evidence that parasitism in eggs of *M. bivittatus* became moderately high at 14 per cent in 1949, the year noted above as the third in a series of high abundance of eggs of *M. m. mexicanus*. The abundance of *M. bivittatus* in Saskatchewan declined to a low level in 1951, and the percentage parasitism also declined. There was another rather striking instance of parasitism in *M. bivittatus* (Table I), in the district of Carmangay, Alta., in 1950, where 26 per cent of the eggs were affected. A substantial portion of whatever infestation is present in the Carmangay district usually consists of *M. bivittatus*, and 1950 was the second consecutive year of greater-than-usual abundance of host adults.

In Western Canada, *Cammula pellucida* eggs are seldom infested by *Scelio* sp(p). In Saskatchewan, a few parasites were found in this host in 1948. The single outstanding record is that of 10 per cent parasitism in eggs from the Red River Valley in 1951. This was at the end of the third consecutive year of abundance of the host in this area.

Discussion

A large total number of grasshopper eggs were examined in these investigations, sufficient to form the basis of certain generalities about scelionid parasites in the economic grasshopper species in Western Canada. Unfortunately, low abundance has often resulted in sketchy or completely deficient representation of some areas, in some years, in the host egg samples. This detracts from the basis of some other generalities, which are nevertheless tentatively offered, pending more thorough and exacting investigations.

The data suggest that scelionid parasites of grasshopper eggs are dependent for success (not merely in the numerical but in the proportional sense) upon the density of the host, since the highest records of parasitism were usually taken after two or three continuous years of grasshopper outbreaks. It is evident that, if a period of host abundance is a prerequisite, and if the same parasite affects all hosts, the predominant species in the grasshopper population should not have to remain the same from year to year during the period.

There is also evidence that, in the Prairie Provinces, warmer local climates favour relatively high parasitism. For example, higher records have been taken in southwestern Alberta and the Red River Valley of Manitoba. These areas lie respectively on the extreme southwestern and southeastern edges of the Great Plains in Canada. According to climatological maps prepared by Hurd and Grindley (1931) the Red River Valley annually enjoys more frost-free days and

has a slightly higher mean summer temperature than most of the Canadian part of the Great Plains. Southwestern Alberta has a similar "warm belt", but it is local and rather sharply defined, and the area from which the high records came seems to be just outside it. It is, therefore, only tentatively possible to explain these instances of relatively high parasitism on the basis of southern latitudes and warmer local climates within Western Canada.

If the observation of more parasitism in eggs of *M. bivittatus* than in *M. m. mexicanus* is significant, and if the parasites are of the same species, or non-specific in their host relations, it may be possible to account for the difference through the egg-laying habits of the host species. In the Prairie Provinces, *M. m. mexicanus* scatters its egg-pods widely through fields of ripening small grain or stubble; *M. bivittatus* often does the same, but in this host there is a marked tendency for the concentration of egg-pods just outside sown fields in certain more exposed, favourable spots. Such concentration may be to the advantage of the ovipositing parasites. This could be regarded as another aspect of density dependence.

Concentration of host eggs, as a factor favourable to the parasite, does not apply to *C. pellucida*, in which the tendency to place many egg-pods in limited areas is very marked. This species apparently establishes egg-beds somewhat like those of *A. cruciata* in Australia, yet, unlike *A. cruciata*, *C. pellucida* is little affected by *Scelio* sp(p). Since eggs of this species are obviously not immune, the reason for the observed comparative rarity of parasitism in them is not clear, again on the assumption that the same parasite is involved. It seems probable that the relatively low parasitism in *C. pellucida* may be explained on the basis of the time of oviposition by the parasite. Table I provides a comparison of parasitism in eggs of *C. pellucida* and of *M. m. mexicanus* collected from the site of a tillage experiment at Davidson, Sask., in which eggs of both were distributed generally throughout the same stubble field in close association and in equal numbers. *M. m. mexicanus* eggs were 9 per cent infested, whereas those of *C. pellucida* were unaffected. An explanation may be found in the fact that oviposition by *C. pellucida* is normally earlier than that by *M. m. mexicanus*. The former usually oviposits in July and August; *M. m. mexicanus* starts to lay eggs in August and continues as long as weather conditions permit.

Summary

In observations on large numbers of eggs of the grasshoppers *Melanoplus mexicanus mexicanus* (Sauss.), *M. bivittatus* (Say), and *Cammula pellucida* (Scudd.) over a 10-year period in Western Canada, the egg parasites *Scelio* sp(p) were not observed to be of critical importance. Among these three hosts, most of the parasitism was found in *M. m. mexicanus* and *M. bivittatus*; there is some evidence that eggs of the latter species harboured slightly more parasites than *M. m. mexicanus*. Eggs of *C. pellucida* are apparently suitable hosts in the physiological sense, but were usually not parasitized.

The percentage parasitism may reach 25 or even 40 per cent in collections from single fields or other limited sites, and thus become of some local importance, probably in the declining phase of a host outbreak. Nearly every instance of outstanding local or general scelionid parasitism occurred at the end of two or three consecutive years of host outbreak. Such a period is about the normal duration of any local outbreak in Western Canada. The percentage incidence of the parasite may, therefore, be dependent upon host density.

It is suggested that the most southerly latitudes of Western Canada, with the longest frost-free period and highest summer temperatures, may favour *Scelio*

sp(p).; this implies that elsewhere in Western Canada the parasite may often mature too late to function at its maximum potential effectiveness; the frequent escape of the eggs of *C. pellucida*, which oviposits relatively early, seems to support the theory of late parasite maturity.

Differences in host egg-laying habits may confer an advantage to *Scelio* sp(p). in attacking eggs of *M. bivittatus*, which often concentrates its egg-pods on limited, favourable spots. This does not apply to *C. pellucida* eggs, which are often densely concentrated in restricted areas.

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The Influence of Food on Cold Hardiness of Insects¹

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Introduction

There is ample evidence that many plants overwintering in cold regions undergo a hardening process in the fall that enables them to survive lower temperatures than they otherwise could (Levitt, 1941). It is generally assumed by entomologists that a similar acquisition of cold hardiness protects those insects that require it. It is the purpose of this paper to examine the evidence that insects develop cold hardiness, and to present new evidence regarding its acquisition and loss.

Insects inhabiting cold climates require some form of protection against low winter temperatures. Though they may escape extremes by virtue of an insulated environment they usually encounter temperatures below 0°C. However, the body fluids of insects undercool to a greater or less extent, usually sufficient to protect against freezing. It is this ability to undercool that confers what we call cold hardiness on the species, except for a small group of insects that can withstand freezing, and that will not be considered in this paper. The remaining majority of species die when ice crystals form in the body; their ability to undercool is therefore a direct and quantitative measure of their cold hardiness.

If a hibernating insect is cold-hardy, there is no reason to assume that its warm-weather stages are not. Often different stages of the species are involved. The overwintering, non-feeding eggs of the grasshopper *Melanoplus bivittatus* (Say) are cold-hardy, but the feeding nymphs and adults are not. When the non-hardy, feeding, summer adult lays its hardy, non-feeding, overwintering eggs, is this *hardening*? When an immobile, non-feeding, hardy, overwintering pupa results from a mobile, feeding, non-hardy larva, is this *hardening*? These examples are comparable to the production of a hardy overwintering seed by a non-hardy annual plant. None of these cases involves hardening, in the accepted sense of the term; instead, the hardiness changes with the stage. Hardening, as a process, cannot be involved when there is a change of stage, though it may follow such change. Hardening is therefore restricted to cases in which warm-weather, feeding forms cease feeding and go into hibernation or in which non-feeding stages increase their hardiness. The former alternative is later shown to be untenable.

As a basis for the work reported herein, it was postulated, on theoretical grounds alone, that ingested plant or insect matter in the digestive tract would freeze at a relatively high temperature and inoculate, or seed, the freezing process in the insect itself. It is known that plant tissues, generally speaking, undercool only a few degrees (Levitt, 1941). It is also known that freshly wounded insects undercool much less than normal ones (Robinson, 1928). The cold hardiness of the tissues of a phytophagous or entomophagous insect, whether great or small, may therefore be nullified by the freezing of its food.

General Methods

Low temperatures were maintained at $-48 \pm 1^\circ\text{C}$. in a refrigerated insulated box. One type of thermojunction-holder was used throughout the experiments. It consisted of an inverted No. 7 cork with a shallow depression cut into the

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narrow end. The thermojunction was centred in this depression, with the copper and constantan lead-wires passing out through the large end of the cork into rubber tubing and thermally insulated near the junction end. A reference junction was maintained at 0°C. in ice-water. Temperatures were read directly from a calibrated scale, the deflection of a sensitive galvanometer being the indicator. With this apparatus the heat of crystallization produces a sudden reversal of the cooling curve (the rebound); the lowest point reached before crystallization begins is the undercooling point.

It has been shown (Salt, 1950) that undercooling points have no validity as *absolute* values unless the time factor is also considered. As the period of undercooling lengthens or as the temperature drops, the chances of ice crystal formation increase. A fast cooling rate therefore results in greater undercooling than a slow rate. Though undercooling points are not fixed, fortunately they are useful for comparison. They offer a convenient means of comparing cold hardness between or within groups, provided the methods used throughout an experiment, particularly the rate of cooling, are uniform.

The rate of cooling was kept constant during these experiments by inserting the thermojunction-holder into a 500-ml. Ehrlenmeyer flask at room temperature and placing them on the metal bottom of the refrigerator. The cooling rate was rather fast, and near the end of a run the cuticle of some of the cutworms used (diameter one-quarter inch) was a few degrees colder than the central gut at any one moment. A slower rate of cooling would have been preferable but the extra time involved was prohibitive. Nevertheless, since the technique remained uniform the results are reliable for comparative purposes. Some discrepancy may be involved if specimens of greatly different diameters are compared. The hardness values for *Ephestia kuehniella* and *Agrotis orthogonia* larvae given below are comparable within species but not between species. In neither are the results correctly placed on the temperature scale.

Many feeding insects regurgitate when disturbed; because external liquids inoculate the freezing process and produce abnormally high undercooling points, those specimens likely to regurgitate were anaesthetized in carbon dioxide and dried, if necessary, before being frozen. In a few cases specimens were frozen in 95 per cent alcohol, which, by removing surface moisture, eliminated the danger of inoculation. As these insects did not survive freezing, this method was used only as a last resort, or when survival was not an advantage. Great care is necessary to ensure that contact moisture does not interfere with undercooling. In very active insects, in fragile ones, and in those forms that are normally moist, it is very difficult to eliminate surface moisture throughout a determination. However, experience with this problem soon enables the operator to detect affected results. Unfortunately a great deal of information published before 1936 is open to suspicion because the effect of contact moisture was not recognized. Evidence of "hardening" may, in some cases, be traced to the avoidance of contact moisture as a result of a slight difference in technique introduced by the "conditioning factor", usually dehydration or low temperature.

By removing specimens from the thermojunction-holder within a few seconds after the undercooling point was reached, ice formation was kept to a minimum, and most specimens survived. In such a short time they did not become hard. The process was not without ill effects, however, for after a few such treatments most specimens became moribund and were incapable of feeding or moulting. Some survived 10 to 12 freezing tests during a period of about a month, during which time they fed and moulted. It is important to realize, however, that survival was a laboratory artifact; had even a minute been allowed

for freezing to proceed at the undercooling temperature, all specimens would have died.

Death does not affect the position of the undercooling point for some time; repeated freezings of a dead *Agrotis* larva over a period of a few hours gave readings within 2°C. It was thus possible to make redeterminations if contact moisture accidentally eliminated the rebound.

Results

Effects of Plant Food

Larvae of the pale western cutworm, *Agrotis orthogonia* Morr., were used as examples of phytophagous insects. They were reared in the laboratory on wheat sprouts. Larvae of the third to sixth instars were frozen either in the mid-instar feeding conditions or else after leaving off feeding in preparation for moulting (premoult condition). A few were also frozen shortly after moulting and before being fed.

The difference in hardness between the feeding and non-feeding larvae (Table I) was so striking that statistical tests of significance were considered unnecessary. The ranges of undercooling scarcely meet, and the means are widely separated.

TABLE I
Cold hardness of feeding and non-feeding *Agrotis orthogonia* larvae.

	No. of larvae	Undercooling points	
		Range, °C.	Mean, °C.
Mid-instar, feeding.....	28	- 6.9 to -15.4	-10.3 ± 2.1
Premoult, non-feeding.....	32	-16.6 to -29.6	-23.6 ± 3.6
Freshly moulted, non-feeding.....	10	-15.2 to -26.2	-20.2 ± 1.1

In addition, some larvae were reared individually and frozen repeatedly at critical times. The undercooling points were high during the feeding period but dropped soon after the larvae left off feeding before moulting. They remained low during ecdysis and until the first food in the new instar was ingested, at which time they rose abruptly. This cycle was repeated in each instar tested. Three typical examples are given in Table II.

TABLE II
Changes in the cold hardness of three *Agrotis orthogonia* larvae during growth and development. Undercooling points in °C.

	Larva 1	Larva 2	Larva 3
3rd instar, feeding.....	-	-	-11.6
" " , premoult.....	-	-	-22.1
4th instar, feeding.....	- 9.1	- 9.0	-11.9
" " , premoult.....	-19.2	-17.6	-22.0
5th instar, feeding.....	- 9.3	- 8.4	- 8.1
" " , premoult.....	-25.2	-19.2	-
6th instar, feeding.....	-	- 9.6	-
" " , premoult.....	-	-26.2	-

Effects of Insect Food

Predatory insects were not available when these experiments were carried out. Consequently *A. orthogonia* larvae were forced to eat insect food by depriving them of any other kind. They would not attack living prepupae of *Ephestia kübniella* or ones that had been freshly killed by heat, but fed fairly readily when the larvae were cut open.

The effect of the insect food on the cold hardness of the host was the same as for plant food. The undercooling points of freshly moulted larvae jumped from below $-20^{\circ}\text{C}.$ to between -8° and $-14^{\circ}\text{C}.$ after feeding. Only one larva moulted successfully after its insect diet. This specimen had an undercooling point of $-24.6^{\circ}\text{C}.$ after moulting to the fourth instar. When fed on *E. kübniella*, its undercooling point rose to $-13.0^{\circ}\text{C}.$; moulting to the fifth instar, the point dropped to $-21.6^{\circ}\text{C}.$, and after further feeding on *E. kübniella* rose to $-7.8^{\circ}\text{C}.$

Effects of Dry Food

Larvae of the Mediterranean flour moth, *Ephestia kübniella* Zell., were used as examples of insects feeding on very dry food that will not by itself freeze or inoculate freezing in the insect. The moisture content of the whole-wheat flour used as food was 7.8 per cent. Larvae ranging in size from one-third to two-thirds grown were selected from a large colony in either the premoult or the mid-instar condition. Premoult larvae are readily recognizable, as the stretching of the integument results in the head capsule and the cervical shield becoming widely separated; in mid-instar larvae they are contiguous.

As in the case of the phytophagous cutworms, the undercooling ranges are distinct (Table III).

TABLE III
Cold hardness of feeding and non-feeding *Ephestia kübniella* larvae.

	No. of larvae	UNDERCOOLING POINTS	
		Range, $^{\circ}\text{C}.$	Mean, $^{\circ}\text{C}.$
Mid-instar, feeding.....	41	-8.7 to -14.9	-11.0 ± 1.6
Premoult, non-feeding.....	41	-16.9 to -25.9	-20.5 ± 2.3
Freshly moulted, non-feeding.....	7	-17.3 to -23.3	-20.2 ± 2.3

A few larvae were tested twice, once while feeding and later in the premoult condition. The results conformed to those of Table III.

Hardiness in *Agrotis* and *Ephestia* larvae is seen to be cyclic. The decrease in hardiness accompanying feeding after the moult is abrupt, whereas the increase associated with cessation of feeding appears to be more gradual. This, together with the possibility of overlap involved in classifying larvae as mid-instar or premoult, probably accounts for the few cases where the undercooling ranges meet or almost meet. In *Ephestia* this point was checked by retaining mid-instar larvae after cooling; those with the lowest undercooling points moulted first, indicating that they had probably ceased feeding before being cooled, even though they appeared not to have reached the premoult condition.

Effects of Starvation

In the early stages of this work it was thought that starving insects might empty their digestive tracts and become cold-hardy. This is not the case in

either *Agrotis* or *Ephestia* larvae, however, for reasons that probably apply to insects in general. *Agrotis* larvae that had been starved to death had bits of wheat sprout tissue from their last meal in the mid-gut, as well as a great deal of unidentified, granular, semi-fluid material. Their crops were full or partly full of a thin, clear liquid with a greenish or yellowish tinge. The hind guts contained grey or brown granular, semi-fluid material. Although in these larvae the fat body was completely used up and the haemolymph volume greatly reduced, the digestive tract contained much liquid and some solid matter, including parts of the last meal.

Whether or not the gut contents were responsible, starved mid-instar larvae did not become more cold-hardy.

Effects of Vegetable Implants

The advantages of surgical operation in this work were recognized soon after its initiation. The drawback was the knowledge that wounding an insect reduces undercooling. Regurgitation, secretion, or excretion of watery fluids by the insect has the same effect. It was this that led the writer (1936) to the discovery that contact moisture on the external surface of an insect was likely to inoculate freezing in undercooled internal tissues. On the other hand, when biological tissues are subjected to drying they ultimately reach a point where the residual moisture, still appreciable in quantity, cannot be frozen.

It was therefore reasoned that the decrease in undercooling caused by wounding could be counteracted by drying so that surface moisture was removed entirely and the underlying tissue was, locally, dehydrated to the point of unfreezability. This was tested and found correct. Dehydrating a wound by holding it against a hot metal lampshade removed surface moisture and its effect on freezing. In this way a larva could be bisected and the original hardiness retained by both halves. By means of ligatures and localized dehydration, an implantation technique was successfully developed.

A premoult fifth-instar *Agrotis* larva was frozen and found to have an undercooling point of $-28.5^{\circ}\text{C}.$, which is in the cold-hardy range (Table I). It was then ligatured at the first thoracic segment, the head amputated, and surplus tissue anterior to the ligature trimmed off. The incision and ligature were then dried and the specimen re-frozen. Its cold-hardiness was retained, the undercooling point being $-26.6^{\circ}\text{C}.$ The cuticle was then slit just behind the ligature, and a piece of wheat sprout, one-eighth of an inch long and one-sixteenth of an inch in diameter, was inserted into the body cavity. A second ligature was then applied at the third thoracic segment, behind the incision, and all tissues lying anteriorly were cut away. The implant, readily visible because of its green color, lay ventrally in the first two abdominal segments. As a result of this treatment, the undercooling point moved up to $-12.5^{\circ}\text{C}.$, into the range of feeding larvae. Two ligatures were then applied, on the third and fourth abdominal segments, and the larva was cut in two between them. The incisions were dried and each piece was frozen. The anterior piece containing the implant was non-hardy ($13.6^{\circ}\text{C}.$), but the posterior piece retained the hardiness of the original larva ($-26.0^{\circ}\text{C}.$). As a further check a similar implant was placed in the posterior piece, which thereupon became non-hardy ($-12.6^{\circ}\text{C}.$). Throughout the experiment the various pieces remained alive.

The above experiment is readily reproducible provided care is taken with the ligatures. Ligaturing causes distension of the body by placing the body fluids under pressure. This is likely to keep the tissues under a ligature knot

moist, even though they were once heat-dried, and to result in inoculation. Some relief of pressure by bleeding is therefore desirable for the best results.

Effects of Dry Implants

Because of the anomalous results obtained with *Ephestia*, in which dry food decreased hardiness, the effects of implants of dry materials were tested. Flour, glass, or cork was implanted into premoult and freshly moulted *Agrotis*, *Ephestia*, and *Loxostege* larvae and into *Agrotis* and *Ephestia* pupae. In every instance undercooling was greatly reduced. In other words, unfreezable implants decreased cold hardiness just as freezable wheat tissues did.

Discussion

The above experiments show that feeding insects may exhibit a cyclic cold hardiness. The non-feeding periods of an instar are characterized by relative hardiness and the feeding periods by relative non-hardiness. A partial explanation can be based on the presence of freezable food, which, by freezing at a higher temperature than insect tissues, inoculates them. The non-hardiness of starved *Agrotis* larvae could be covered by such an explanation since food is retained in the gut, but the non-hardiness of feeding *Ephestia* cannot. In *Ephestia* the dry food cannot inoculate freezing, but the digestive juices moistening the food may be responsible. However, the wheat-sprout implants in *Agrotis* decreased hardiness without regard to digestive juices. In this case, the effect was caused by the inoculation of the hardy insect by the less hardy wheat sprout, or by the mere presence of the implant, or by both. The dry implants of flour, glass, and cork could not inoculate by their own freezing; therefore, their effect was due solely to their presence.

It appears, then, that insect tissues react to the presence of foreign matter, such as implants, and that one effect of the reaction is a reduction in cold-hardiness. By analogy, food is in the same category if we consider, as there is much justification for doing, that food in the alimentary tract is foreign to the insect's tissues. Even digestive juices, after secretion, may be considered as foreign matter. The hardiness of the liquid material observed in the mid-gut of *Agrotis* during the moult, when the insect is hardy, may be explained if at such times digestive secretions have ceased. The material is a residue that is no longer foreign to the insect's tissues, since digestive juices are no longer being secreted into it and are probably absent.

Insect food, then, may reduce the cold-hardiness of an insect by direct inoculation or else as a result of a reaction by the insect's tissues to its presence as foreign matter.

Although the present experiments deal only with developing larvae, the results probably apply equally to nymphs and adults. Feeding adult insects should be relatively non-hardy except just after emergence (unless the meconium inoculates) or during the periods of voluntary fasting, if the gut is emptied.

As a result of these experiments it is necessary to revise the concept of insect cold hardiness. A distinction must be made on the basis of the means whereby a change in hardiness is effected. The change may be more or less abrupt, as when a change of stage is involved, or it may be gradual as a result of the effect of an ecological factor. The degree of hardiness possessed by a newly laid egg, for example, is basically inherent or characteristic of the species. It may have been indirectly affected by environment via maternal physiology, but any such variation is secondary, deviating only from a primary and fixed degree of

inherited hardiness. On the other hand, if a change of hardiness occurs in the egg after oviposition it is probably a result of ecological factors.

It is proposed, therefore, to make a distinction on the basis of whether hardiness is changed abruptly as, for example, by cessation of feeding or change of stage, or gradually as a result of environmental factors. The first type is possessed by every insect to a fairly fixed degree; it may be considered a property. The second type is *developed*, and is additional to the basic or inherent hardiness. Having been developed, it may also be lost, though possibly not below the basic level.

The verb *to harden*, and its forms, and the noun *hardening* are applicable only to the second type of hardiness. Thus an insect may be hardy, yet may not be able to *harden*. The two aspects of the problem, then, are *hardiness* and *hardening*. They are entirely different and should not be confused.

Increased cold hardiness associated with a change of stage is not hardening. The apparently increased cold-hardiness of a phytophagous larva when it becomes premoult is not hardening. The larval tissues were just as hardy when the larva was feeding, but the food nullified much of their hardiness. The larva became less hardy as a result of feeding, rather than more hardy when not feeding; certainly it did not *harden*. Each stage of every insect, then, whether feeding or non-feeding, has a basic cold hardiness. Hardening, if it exists at all, proceeds from this point.

A review of the literature on insect cold hardiness reveals the strong influence exerted by prior work dealing with the experimental cold hardening of plants. Most of the entomological papers deal with cold hardiness during hibernation as compared with that found in warmer weather. Most, if not all, of the differences that were found are now interpretable on the basis of food. Where the comparisons were made from time to time as the insect entered or emerged from hibernation, some of the data show the changes to be gradual. Although the following explanation may not be completely satisfactory, it seems likely that the samples of insects used in the determinations would be a mixture of hardy and non-hardy as hibernation approached and receded; thus the *average* hardiness would show a gradual change by virtue of a changing proportion of hardy and non-hardy individuals.

Decker and Andre (1936) determined the cold hardiness of chinch bugs in the late winter and early spring. They state that their data indicate that bugs collected early in the season (January) are more uniform than those collected later (April 1). They further state, "The bugs collected April 1 were fairly active at the time the collection was made, and it is entirely possible that they had imbibed some moisture or plant juices which would account for their greater susceptibility to low temperatures". Payne (1927), working with larval oak borers, *Synchroa punctata* Newm. and *Dendroides canadensis* Lec., stated, "Artificial softening was accomplished by high temperatures and high humidity plus food. Attempts to soften by high temperature alone were unsuccessful, since the larvae dehydrated beyond their vital limits". Food was no doubt the responsible factor. Such examples show that increased cold hardiness was taken to indicate that hardening had taken place.

The problem of whether cold hardening actually occurs in insects is a difficult one that has not as yet been satisfactorily solved. Much of the evidence is no longer pertinent as a result of the division of the problem made above. In addition, at least that part of the evidence in favour of hardening that was based on water loss preparatory to hibernation is attributable to cessation of feeding.

Most earlier workers regarded the decreased moisture content of hibernating forms as a prime cause of hardening. There is usually a good inverse relationship between insect cold hardiness and moisture content when summer and winter forms are compared, but no interdependence is thereby proved. Regardless of hardiness, a decreased water content is to be expected in hibernating insects because: (1) The contents of the fore- and mid-guts of feeding insects are likely to be more moist than insect tissues, being either moist food plus digestive juices or dry food plus digestive juices. When the gut is emptied preparatory to hibernation the absolute moisture content is decreased and probably the percentage moisture content also. (2) The storage of fat as a prelude to hibernation may be expected to replace moister tissues. (3) Haemolymph volume is often reduced before hibernation.

The gut contents affect hardiness, not hardening. To show that decreased moisture content causes cold hardening, more direct proof than a mere correlation is necessary. The same applies to the possibility of ecological causative factors. At present the evidence is confused, and it is not even certain that hardening occurs in insects.

Summary

It is postulated that the cold hardiness of feeding insects is decreased by the presence in the digestive tract of less cold-hardy food, which freezes at a higher temperature and inoculates freezing in the insect tissues.

Feeding larvae of *Agrotis orthogonia*, fed on wheat sprouts, were much less cold-hardy than non-feeding premoult or freshly moulted larvae. The same was true when the larvae were forced by starvation to feed on macerated larvae of *Ephestia kühniella*.

Feeding larvae of *E. kühniella*, fed on whole-wheat flour, itself unfreezable, were much less cold-hardy than premoult and freshly moulted larvae.

Starvation of mid-instar *Ephestia* and *Agrotis* larvae did not result in increased hardiness, possibly because the alimentary tract retained its contents.

When pieces of wheat sprout were implanted into the body cavities of hardy premoult *Agrotis* larvae, hardiness was decreased to the level of feeding larvae. By ligaturing such larvae and excising the implants, hardiness was restored to the original level.

Dry implants of flour, glass, or cork decreased undercooling of hardy *Ephestia* and *Agrotis* larvae and pupae. The effect is attributed to their status as foreign bodies.

The action of food in decreasing cold hardiness may also be attributed to its classification as foreign material. Moist foods may reduce cold hardiness by direct inoculation if they are less hardy than the insect tissues, but their effect as foreign material probably overrides such action. Digestive juices may also be considered foreign matter after they have been secreted into the alimentary tract.

It is important to distinguish between an abrupt change of *hardiness* resulting from a change of stage or cessation of feeding, and the gradual *hardening* of a non-feeding insect resulting from the operation of an ecological factor. Cold hardiness is possessed by every insect, to a fairly fixed degree, as a property; hardening, if it occurs at all in insects, proceeds from this point. The evidence in favour of hardening in insects is at present in an unsatisfactory state. Its relationship to reduced moisture content is not settled and much of the earlier evidence can be rejected because it compares feeding and non-feeding insects.

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A Method of Tagging Prairie Mosquitoes (Diptera: Culicidae) with Radio-Phosphorus¹

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Bugher and Taylor (1949) and Hassett and Jenkins (1949) reared highly radio-active adults of *Aedes aegypti* (L.) from larvae kept in a solution of radio-active phosphorus. Jenkins and Hassett (1949) obtained radio-active adults of *Aedes pullatus* (Coq.) and *Aedes excrucians* (Wlk.) with an average radio-activity of 4,300 counts per minute by rearing larvae in ponds to which radio-active phosphorus was added. Jenkins and Hassett (1951), with sub-Arctic mosquitoes, chiefly *Aedes communis* (Deg.), produced adults with radio-activity from 100 to 3,770 counts per minute from larvae reared in vats of P³² solution; adults released and recaptured in the field had an average radio-activity of 915 counts per minute. Thurman and Husband (1951) tagged larvae with P³² and released 400,000 adult mosquitoes in California. Of this number, 249 adults were recaptured at distances up to 1 1/2 mi. downwind and 1 1/2 mi. upwind from the release point. Yates *et al.* (1951) and Hassett and Jenkins (1951) produced radio-active adults of *Aedes sticticus* (Meig.) and *Aedes aegypti* (L.) by allowing them to feed on P³² solutions; the radio-activity was readily detectable with a Geiger counter for as long 13 days. Hassett and Jenkins (1951) in a series of experiments with *Aedes aegypti* (L.) found that there was a 38 per cent mortality when late third- and early fourth-instar larvae were allowed to complete development in a 0.25- μ c.-per-ml. solution of P³² at a population of two larvae per millilitre of solution; but with larvae reared in a 0.1- μ c.-per-ml. solution at the same population no mortality was recorded. In experiments with *Simulium* spp. Fredeen *et al.* (1952) obtained tagged adults by keeping larvae in a 0.2- μ c.-per-ml. solution of radio-phosphorus in the form of P³²O₄ for 23 hr.; after immersion in radio-active phosphorus the larvae were transferred to a non-radio-active medium, where they were reared. This new technique was more practical than those tried before, as it increased the productivity of the equipment and man-power, and conserved radio-active material.

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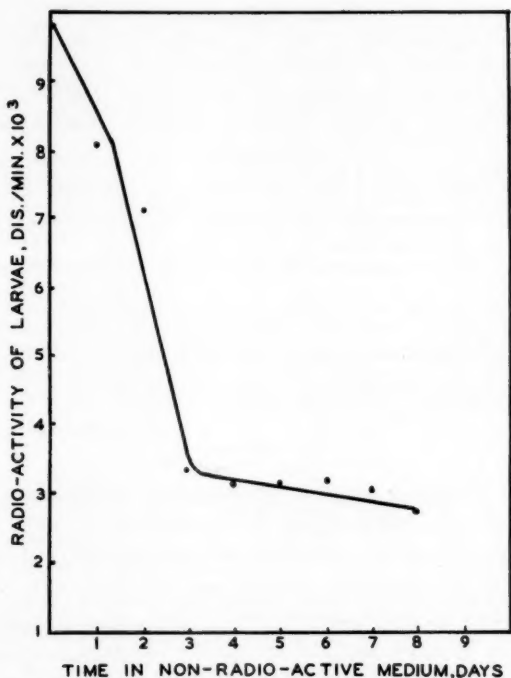


Fig. 1. Rate of loss of radio-activity in mosquito larvae when introduced into a non-radio-active medium for development.

Early in the spring of 1951 a laboratory experiment was conducted with prairie mosquito larvae to determine the optimum concentration of radio-active phosphorus required to treat the maximum number of larvae per unit volume of radio-active medium, with a 24-hr. exposure period, for the production of readily detectable radio-active adults. One hundred fourth-instar larvae, mostly *Aedes spencerii* (Theo.) and *Aedes campestris* D. & K., were treated in separate aquaria with 100 ml. of solutions at concentrations of 0.025, 0.05, and 0.1- μ c.-per-ml. of radio-active phosphorus ($P^{32}O_4$). The larvae were exposed to the radio-active solutions for 24 hr., and then were transferred into aquaria containing slough water for development to the adult stage. The average radio-activity in the larvae taken immediately after the exposure period from their respective solutions was 5,100, 9,500, and 42,700 dis. per min. Adult mosquitoes that emerged approximately five days after the exposure period had an average radio-activity of 1,300, 2,700, and 6,620 dis. per min., respectively.

These results indicated that tagged adults could be obtained from fourth-instar mosquito larvae kept for 24 hr. in a 0.1- μ c.-per-ml. solution of radio-phosphorus at a density of one larva per millilitre of radio-active solution, that after exposure the larvae could be reared in a non-radio-active medium, and that the adults would contain readily detectable amounts of radio-activity.

A field experiment was conducted at Dundurn, Sask., to determine whether laboratory results were applicable to field conditions. At the time of this experiment a mixed population of fourth-instar larvae of *Aedes campestris* D. & K.,

Aedes spencerii (Theo.), *Aedes flavescens* (Müll.), and *Aedes dorsalis* (Meig.) was available from the spring flood pools.

Fourth-instar larvae were readily collected in the vicinity of the experimental site, and were tagged in 50-litre waxed, galvanized tubs. The concentration of P^{32} used throughout the experiment was approximately 0.1 μ c. per ml. The larvae were immersed in the radio-active solutions for 24 hr. and then placed in an open-top rearing cage in water in a natural mosquito habitat. The cage was 1 yd. by 1 yd. by 1 yd. with the sides and bottom made of 28-mesh plastic screening. Before each lot of larvae was placed in the rearing cage an aliquot of six or more larvae was removed and washed in slough water for a few minutes, and their radio-activity determined. An immersion period of 24 hr. was used for 11 lots tagged; for five other lots this period had to be increased to 48 hr. because inadequate amounts of radio-activity were absorbed. It was estimated that 470,000 healthy larvae were tagged and placed in the rearing cage for development and dispersal. The radio-activity of all larvae after immersion showed an extreme range from 4,200 to 35,000 dis. per min., but most were in the range from 7,500 to 12,000 dis. per min.

Approximately 500 field-activated larvae were placed in a tub of slough water, and six were removed each day to determine the rate of loss of radio-activity while in the non-radio-active medium. The larvae lost approximately 50 per cent of their original radio-activity in the first two days after exposure to P^{32} . After this the rate of loss was considerably lower (Fig. 1).

Adults reared in a non-radio-active medium from larvae tagged in the field had an average radio-activity of 2,170 dis. per min. Adult mosquitoes taken from the walls of the field rearing cage within a few hours of emergence had an average radio-activity of 2,130 dis. per min.

TABLE I
Data on Three Radio-active Mosquitoes Collected in the Field

Species	Sex	Date, 1951	From Release Point		Radio-activity dis., min.
			Distance yd.	Direction	
<i>Aedes spencerii</i>	♀	June 5	25	W	1,800
<i>Aedes sp.</i>	♀	June 9	550	SW	1,200
<i>Aedes flavescens</i>	♂	June 21	150	NNE	1,600

TABLE II
Data on Other Radio-active Flies Collected in the Field

Species	Sex	Date, 1951	From Release Point		Radio-activity dis., min.
			Distance	Direction	
<i>Hydrotaea meteorica</i> (L.)	♂	June 9	350	SE	1,800
<i>Gynnodia</i> sp.	♀	June 15	500	SE	1,600
<i>Gynnodia</i> sp.	♀	June 15	300	SE	1,600
<i>Pyrellia serena</i> (Meig.)	♀	May 31	550	SE	2,700
<i>Hydrotaea</i> sp.	♀	June 11	1,000	NNE	1,100
<i>Bufo lucilia silvarum</i> (Meig.)	♀	June 11	1,000	NNE	3,000
<i>Protophormia terranova</i> (Desv.)	♂	June 9	350	SE	4,500
<i>Protophormia terranova</i> (Desv.)	♂	June 9	350	SE	6,000

Approximately 418,500 adults were collected by means of two New Jersey mosquito light traps and by sweeping around livestock, around observers, and through vegetation, in a systematic order about the release site. Three radio-active mosquitoes and eight other radio-active flies were captured (Tables I and II).

It is assumed that the cyclorrhaphous flies (Table II) became radio-active through feeding on the solutions that were used for radio-activating mosquitoes, as these flies are not predators and have no aquatic life stages.

These experiments indicate that tagged adult mosquitoes can be produced by keeping fourth-instar larvae in a 0.1- μ c.-per-ml. solution of P^{32} for 24 hr. at a density of one larva per millilitre of radio-active solution, and then allowing the tagged larvae to complete development to the adult stage in their original or normal habitat. The short exposure method reduces the possibility of over-exposure to surface irradiation, and reduces mortality caused by prolonged artificial rearing conditions. There is also less chance of the larvae assimilating lethal doses of the radio-phosphorus.

This method makes it possible to tag larger numbers of mosquitoes with a minimum of equipment and radio-element, because the radio-active solutions can be used several times simply by adding enough radio-element to the old solution to bring it up to the desired radio-active strength. Furthermore, if the solutions become putrid and probably harmful to mosquito larvae or pupae they can be discarded without the loss of large numbers of mosquitoes and of radio-element.

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